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Dear Mr. Fields:

My colleagues and I appreciate the careful and thoughtful review of our Center application. I would like to take this opportunity to clarify a few issues identified in the review.

1. PROGRAM AS AN INTEGRATED EFFORT AND PROGRAM DIRECTOR

The reviewer notes, "outside of a monthly seminar program and yearly retreat...extensive interactions among Center members have been quite limited, and plans to increase the number of interactions were not elucidated in the application. This is considered a weakness in the integration of the overall Center."

Certain details about interactions among investigators were omitted or limited in the proposal in the interest of brevity. In addition to the monthly seminar and yearly retreat, extensive interactions among investigators occur on the telephone, over email, during informal in person meetings and at formal monthly Executive Committee meetings that precede the monthly seminars. In addition, small scientific workshops (all day or evening meetings) are held monthly and large scientific workshops (two days or more) are held approximately three times per year. Further collaborative investigations have been formed beyond the scope of work in the Children's Center during these meetings and interactions. Dr. Frank Gilliland has had a leadership role in the planning and execution of these events.

2. ADMINISTRATIVE CORE

Reviewer 1 comments that "little information on organization regarding the effectiveness of the Administrative Core in the previous funding period was provided."

We provided limited information on the administrative core in the previous funding period in order to focus on the organization of the redesigned Centers program. In light of the summary by Reviewer 1, it is apparent that we did not present sufficient clarity for this reviewer to appreciate structure and organization of the Administrative Core. The Administrative Core governs the Executive Committee, the External Advisory Committee, and the administrative staff. The Community-Based Participatory Research (CBPR) Advisors are not directly linked to the Administrative Core; rather, they advise the CBPR project which is guided by the overall Administrative Core. The Executive Committee functions in essence as an internal advisory committee. It therefore does not have community representation or CBPR representation

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District (Dr. Ospital). This is the lead air quality agency in Southern California, an area in which air quality has considerable political importance. We should also point out that the COTC has a large advisory committee that will meet annually. This committee includes the community-based members of the CBPR Steering Committee, and the progress of the CBPR will be reviewed, and advice will be sought from the full group of COTC advisors. At each of these meetings, a two-hour scientific session will allow for further explanation of the public policy and burden of disease implications of the research. Although the COTC advisors contain numerous leaders in their fields (environmental rights, asthma, etc.), we can appreciate the usefulness of additional members of the COTC, and we have begun a search for community leaders from the political arena (such as elected officials with an interest in air pollution).

The Committee Budget Recommendations section notes that the budget for Ms. Hricko and Dr. Kuenzli needs to be clarified because "they appear to have a similar requested budget in the COTC." Dr. Kuenzli and Ms. Hricko will contribute both to Project 1 (the CBPR project) and to the COTC, and the distribution of tasks and budget reflect the complementary goals and integrated activities of these components of the Center, an integration we consider to be a strength. Both components make use of common resources, such as the Children's Health Study video, which these investigators will also contribute to revising. However, there is neither duplication nor overlap of budget. In order to clarify the tasks of each investigator in the COTC and CBPR, we have prepared the attached table, which we believe demonstrates that the specific activities within and between each component of the Center are quite distinct.

3.2. Project 2: Pollution-Enhanced Allergic Inflammation & Phase II Enzymes

Reviewer 1 states the following:

> *...the principal investigator frequently refers to antioxidant properties of the 'sentinel' GST and NQO1 genes when relating this Project to the other Research Projects that focus on oxidant gasses. The enzymes produced by transcription and translation of these genes are normal biochemical catalysts, with specific substrates, and the products of oxidative stress like superoxide, hydrogen peroxide, lipid peroxides, and hydroxyl radical are not substrates for these enzymes. It is not likely that the activities of the Phase II enzymes, that are the focus of this Project, would affect the process of oxidative stress when it is caused by O_3 or other oxidative gasses. Thus trying to make sweeping conclusions about the susceptibilities of asthmatic children, who may have reduced responsiveness to the induction of the Phase II enzymes, would probably not be applicable when the cause of the adverse effects is exposure to oxidant gasses* (p. 12, 2nd paragraph).

As the reviewer notes, we would predict that the greatest effects of the GST and NQO1 genes would be on diesel exhaust particles, environmental tobacco smoke and the like, in which the gene products can detoxify the relevant substrates. However, we believe that these genes are relevant to gaseous pollutants. It should be noted that there are now multiple studies that show that polymorphisms in these genes may play a role in determining susceptibility to ozone. For example, Romieu at al. (Thorax 2004 Jan;59(1):8-10) reported that asthmatic children with a genetic deficiency of GSTM1 (i.e . GSTM1 null) had greater ozone related decline in FEF(25-

75) than those with a functional form of the gene and that this deleterious effects of ozone on the small airways could be mitigated by antioxidant supplementation. David et al., (Am J Respir Crit Care Med. 2003 168:1199-204) showed that in GSTM1 null children there was a significantly reduced risk of asthma in those bearing the functional form of the NQO1 gene. The reason for these results is that the antioxidant response pathway is complex and inter-related, thus Otto-Knapp et al., (Inflamm Res. 2003 52:51-5.) demonstrated that in nasal tissue biopsies incubated with 120 ppb ozone, GSTM1-deficient patients showed a significantly enhanced upregulation of SOD activity. Similarly, Corradi et al., (Toxicol Lett. 2002;134:219-25.) have reported that controlled short-term exposure to ozone induced changes in biomarkers of lung inflammation and oxidative stress in exhaled breath condensate and that these changes were dependent on GSTM1 and NQO1 polymorphisms.

Additionally, it should be realized that NQO1 and GSTM1 are chosen as sentinel antioxidants as a proof of concept. As important genes controlling responses to ozone are identified by the other projects and the general scientific community, they can be incorporated in our study.

> *A suggestion for this study is to address the question of how Phase II enzymes and Th2 cytokines work together, or whether Th2 cytokines release is triggered by Phase II enzymes?* (p. 13, 4th paragraph).

This is a judicious suggestion. We will analyze our results to see if correlations between Th2 cytokines and Phase II enzyme expression exist in nasal lavages and sputum samples.

> *Dr. Diaz-Sanchez's laboratory and appointment are at the University of California, Los Angeles (UCLA), whereas the Center is based at USC. Although the facilities at UCLA are certainly adequate for the conduct of these studies, no details are presented in this Project or the Center application about how the investigator will interact on a regular basis with other members of the Center, outside of the monthly Executive Committee meetings. It is possible that the "research focus groups" will provide a forum for scientific exchange, but no details were provided about the specific nature of these groups, or whether Dr. Diaz-Sanchez or members of his laboratory, attend these groups* (Critique 1: Environment).

Although Dr. Diaz-Sanchez' laboratory is based at UCLA (only 30 minutes from USC), he is in attendance at all Executive Committee meetings, regularly attends seminars, research dinners and meetings, and is in direct contact with other Center investigators via phone and email. The Center Administrator notifies all Center members, including those outside of USC and UCLA, of all upcoming meetings, activities, and events, and provides a forum for virtual scientific exchange.

3.3. Project 3: Air Pollution, Exhaled Breath Markers & Asthma in Susceptible Children

Reviewer 1 states:

Reviewer 2 also notes, "It is not predictable to what extent these initiatives will lead to sustainable developments. The list of potential activities is very long, and it cannot be assessed which one will be successful and sustainable" (p. 20, 8th paragraph). The CEHC's COTC appreciates the reviewers' concerns about sustainable development and the COTC is spending a great deal of effort in developing initiatives that it hopes will lead to sustainable developments. The list of potential activities, although long, is one that has been carefully conceived and that the director and Center investigators believe will add to the possibility of a future Los Angeles that will better protect children in an environment where the automobile and other mobile sources of pollution will be better controlled. We refer the reviewer to the article on air pollution policy and sustainable development suggestions "Breathless in L.A.: the Exhausting Search for Clean Air" (Künzli et al., 2003, *Am J Pub Health, 93(9),* pp. 1494-1499) for ways to reduce air pollution and protect children's health.

5. FACILITY/SERVICE CORES (Exposure Assessment and Modeling)

> *Diesel exhaust particles and PM2.5 that are part of Research Project 1 [should have been 2] are not addressed in this Core...they argue that measurement of particulate matter would be 'unduly expensive, logistically problematic, and effectively infeasible within the budgetary limitations' for this Center application...investigators ignore a potentially exciting opportunity to provide relevant data for a third of the Center* (p. 21-22).

Project 2 is a mechanistic project that uses controlled exposure to diesel exhaust or diesel exhaust particles. We agree that knowledge of recent exposure to diesel would be useful. However, there are currently no reliable markers for exposure to diesel exhaust. A proxy using exposure to $PM_{2.5}$ or elemental carbon might be worthy of consideration, but this would necessitate measuring personal exposure to particles. Such an undertaking would entail a significant financial and labor commitment to active filter collection and subsequent laboratory analyses, beyond the limits of the available budget. The additional burden this would place on subjects could also be a disincentive for recruitment in these studies, especially for children who would need to wear small backpacks to accommodate pumps and filter sampling instrumentation. Some local PM data is available from neighborhood monitoring stations, and these data will be reviewed to assess potential variability in the respective subjects' communities. Regardless of the baseline exposure of the subjects, each will receive an additional known exposure dose in a controlled setting, and the acute nature of the response will be carefully characterized.

> *It seems possible that they could have included at least a scaled-down measurement of particulate matter to incorporate into their sophisticated models of traffic to provide vitally needed data on the exposure of children to relevant particles* (p. 21-22).

We agree with the reviewer that obtaining PM samples would be highly desirable. We had numerous discussions among the study staff to explore various sampling designs and instrumentation options for monitoring PM levels, including designs for assessing levels at only a subset of locations. Reliable PM sampling equipment is expensive and requires more technician time for deployment than the passive Ogawa samplers we will be using to monitor

NO, NO_2, and O_3. We concluded that the number of PM samplers we would be able to deploy would not yield enough useful information in the context of our study goals to justify the costs. Furthermore, we would have had to scale down our monitoring of NO, NO_2, and O_3 to accommodate the limited PM sampling. This would have compromised our ability to provide comprehensive data for these pollutants, both for direct health analyses and as inputs to our proposed statistical models of exposure. Still, we recognize the value of obtaining PM measurements, and have already obtained separate funding to do limited PM sampling in Long Beach. We plan to seek additional separate funding to perform PM monitoring in other study communities.

> *The case control study (Project 1) has to rely on retrospective exposure assignment, which may introduce some exposure misclassification* (p. 21, critique 1).

This is a good point, and one that we took seriously in designing our sampling plan. To assess the potential misclassification error, we analyzed available pollutant data from our Children's Health Study. Specifically, for each pollutant (NO, NO_2, and O_3), we computed the intra-class correlation (ICC) between two exposure estimates: 1) the multi-year average pollutant level from 1995-2000 and 2) the average computed for the year 2000 using only five specific 2-week periods. The latter corresponds to the proposed sampling plan for this study, while the former corresponds to the type of exposure we ideally would assign to each subject, i.e. their long-term exposure to these pollutants. The ICC's were R=0.90 for NO, R=0.97 for NO_2, and R=0.93 for O_3, indicating that the average derived from limited sampling in 2000 is a very good estimate of long-term average pollutant levels. This finding is also supported by our observation that there have been relatively small fluctuations in annual average pollutant levels in these study communities over the past 10 years. Based on these analyses, we concluded that the degree of exposure misclassification due to retrospective assignment would be relatively small, and should not compromise the power of the study to model pollutant levels and detect health effects.